IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:	Confirmation No: 5701
Pamparana	Group Art Unit: 1614
Application Serial No.: 09/869,333	Examiner: Raymond J. Henley III
Filed: July 26, 2001	Attorney Docket No.: 026392-00095

For: Essential Fatty Acids in the Prevention of Cardiovascular Events

DECLARATION UNDER 37 CFR § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Dr. Benjamin Levinson, declare that:

1. My current position is Director of Clinical Research at Reliant Pharmaceuticals, Inc., a licensee of the above-identified application. My curriculum vitae is attached as Attachment 1.

2. As shown in the following paragraphs, a dose of about 1g/day of omega-3 fatty acids has been reported to not have a clinically significant effect on blood lipid levels, which are risk factors for the occurrence of cardiovascular events. However, surprisingly, a dose of about 1g/day of omega-3 fatty acids does have a significant effect on the reduction of the occurrence of cardiovascular events.

3. The GISSI-Prevenzione trial, as reported in *Lancet*, 354:447-55 (1999), attached as Attachment 2, studied over 11,000 patients who previously suffered a myocardial infarction, to monitor the reoccurrence of cardiovascular events. Patients in the omega-3 fatty acids group were administered omega-3 polyunsaturated fatty acids at a dosage of 1g daily. See the Summary under "Methods," page 447. The study monitored changes in blood lipid concentrations and looked at several efficacy endpoints, including the occurrences of cardiovascular deaths and sudden death from a cardiovascular event. See Table 3, page 450.

4. The changes in blood lipid concentrations at 6 months are shown in Figure 2, page 450. As noted on page 451, compared with baseline values, there were reportedly no clinically important changes in the blood cholesterol profile (total cholesterol, HDL cholesterol, or LDL cholesterol) during this time period, in patients administered 1g/day of omega-3 fatty acids.

5. I independently verified that the changes in blood lipid concentrations reported in the GISSI-Prevenzione trial would not have been expected to affect the risk of cardiovascular events. Risk models, such as Framingham[1] and PROCAM[2] address the 10-year cardiovascular risk associated with current blood lipid levels; that is, these models estimate the risk of a cardiovascular event (specifically, cardiovascular death or non-fatal myocardial infarction) within the next 10 years, based on the blood lipid levels entered into the model. These models are well established and widely accepted, and

[1] The Framingham model is described, for example, in Kannel et al. (1976), *Am J Cardiol* 38: 46-51. An online version of the Framingham model is available at http://www.chd-taskforce.com/framingham.php?iSprache=1&iVersion=1&iSiVersion=0.

[2] The PROCAM model is described, for example, in Assmann et al. (2002), *Circulation* 105(3): 310–315. An online version of the PROCAM model is available at http://www.chd-taskforce.com/calculator.php?iSprache=1&iVersion=1&iSiVersion=0.

are used among both experts in the field and the average physician. Treatment guidelines for dyslipidemia have been based upon such risk models, such as the National Cholesterol Education Program (NCEP) Adult Treatment Panel (ATP) III guidelines, published by the National Institutes of Health.

6. I entered the baseline and 6-month blood lipid concentration data reported in the GISSI-Prevenzione trial into the online Framingham and PROCAM models. With respect to the Framingham model, the data resulted in a predicted 10-year cardiovascular risk of 16% at baseline and at 6-months, meaning that the omega-3 fatty acid treatment had no effect on the 10-year risk of the occurrence of a cardiovascular event. With respect to the PROCAM model, the data resulted in a predicted 10-year cardiovascular risk of 20% at baseline and 21% at 6-months, meaning that, rather than reducing the risk, the omega-3 fatty acid treatment slightly increased the 10-year risk of the occurrence of a cardiovascular event. These results strongly imply a lack of clinical benefit of 1g/day administration of omega-3 fatty acids in reducing the occurrence of cardiovascular events, based on the changes in blood lipid concentrations.

7. This predicted lack of clinical benefit is in stark contrast to the highly statistically significant reduction in cardiovascular events actually achieved in the GISSI-Prevenzione study. The reoccurrence of cardiovascular events is reported on page 451 of the *Lancet* paper. In the omega-3 group, there was a highly significant 30% decrease in cardiovascular deaths (p=0.0242) and a highly significant 45% decrease in sudden death (p=0.010) with the 1g/day administration of omega-3 fatty acids.

8. The reduction in the reoccurrence of cardiovascular events with 1g/day administration of omega-3 fatty acids is surprising and unexpected in view of the prediction from the widely accepted Framingham and PROCAM models that such therapy would be completely ineffective for such a purpose, due to a lack of a clinically significant benefit in normalizing blood lipid levels. I would not have expected a daily dose of essential fatty acids that has no clinically significant benefit in normalizing blood lipid levels to have a significant effect on the reduction of the reoccurrence of cardiovascular events.

9. I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent(s) issuing therefrom.

Benjamin Levinson
Dr. Benjamin Levinson

Oct 30, 2007
Date

BENJAMIN LEVINSON, M.D.
91 DEAD TREE RUN ROAD
BELLE MEAD, NJ 08502
908-359-2536 (CELL 908-910-4815)
blevinson@patmedia.net

CURRICULUM VITAE

SUMMARY

I began my career in Clinical Pharmacology at E.R. Squibb and Sons where I achieved the positon of Associate Director. I have performed an entire cadere of Phase 1-early Phase 2 studies such as pharmacokinetic interaction studies, bioequivalence and bioavailability evaluations. I then continued in Clinical Research being the primary medcial monitor for fosionril (Monopril). I served on the pre-clinical/clinical drug development committies at Squibb/ Anaquest and WellSpring to help identify potential candiadates and weed out new drug candidates before company investment was too great. In this way I have saved a great deal of money and focus precious R&D dollars on only those candidates with a high promise of success. In addition, I have improved the Intellectual Property position of new drug candidates through recognition of new concepts and inventions with patent potential. I am an trained in Internal Medicine with 12 years of pharmaceutical industry experience in Phases 1-4 clinical development. I currently work in the field of lipidology. Previosuly I worked with a hematologically-related metabolic drug, and have worked in the past with anti-hypertensives (angiotensin-coverting enzyme inhibitors, an anti-kaliuretic and an alpha-receptor antagonist), an opioid antagonist, a transdermal analgesic patch, an antiemetic, and the monobactams. Finally, I have extensive experience with electronic data capture as well as its use in clinical studies and its potential for post-approval safety monitoring and compassionate use. Presently, I am employed by Reliant Pharmaceuticals in the capacity of Director of Clinical Development where I am responsible for various programs related to the pharmacological intervention of lipid disorders.

EXPERIENCE

2007 Reliant Pharmaceutical Liberty Corner, NJ

Director, Clinical Development

- Chair two development teams that manage the process of pharmaceutical development and market potential of new and expanded indications of company products
- Provide scientific review of publications related to products in development
- Offer market support through analysis of product and competitive data
- Provide direction for the NDA supplemental submission on the pediatric population and the potential indication for use of the agent in pediatrics.
- Provide guidance in the development of a centralized, 21 CRF compliant computer database for pharmacovigialnce and data-meta analysis

2005 Infacare Pharmaceutical Plymouth Meeting, PA

Vice-President of Drug Development

- Helped in acquisition of a 35 million dollar venture capital investment through scientific presentations to several different firms on both East and West coast.

BENJAMIN LEVINSON, M.D.
91 DEAD TREE RUN ROAD
BELLE MEAD, NJ 08502
908-359-2536 (CELL 908-910-4815)
blevinson@patmedia.net

- Transferred form WellSpring Pharmaceutical, a sister company discussed below, to work exclusively on a new drug candidate. Very aware of the vaule of R&D investment and helped save several million dollars by internailization of key drug development processes (such as clinical monitoring). Wrote the protocols and, with exhaustive work with the FDA, implemented Phases 1-3 clinical trials for stannsoporfin, a new drug candidate, in the field of drug metabolism, specifically hyperbilirubinema. In addition, wrote and monitored the long-term clinical safety program for the drug.
- Responsible for meeting all FDA pre-clinical demands for pharmacosafety and toxicology and supervised the conduct of a portfolio studies directed to fulfill regulatory requirements.
- Supervise the entire corporate project team that includes CMC, pre-clinical and clinical drug development.
- Coordinate the data management, statistical analysis and report writing for the clinical and pre-clinical programs.
- Manage the budget and activities of several consultants in pre-clinical, laboratory, and clinical venues.
- Liaison with key opinion leaders and developed the corporate scientific advisory board. Able to create tremendous value for company in development of Advisory Board for marketing and sales.
- Presented data to both the internal Board of Directors as well as the investment community to attract investors for the company.

2001 WellSpring Pharmaceutical Neptune, NJ

Vice-President of Drug Development

- Spent four years with a primary focus on stannsoporfin. Was responsible for all pre-clinical (pharmacology and toxicology) and clinical drug development (Phases 1-3).
- Supervised all preclinical studies to meet regulatory requirements as well as wrote and conducted a Phase 3 study and the long-term follow-up program. Had all data entered and reports written on all prior studies done under an academic IND and developed a worldwide database for consistent data management.
- Author of an issued patent on the drug manufacture process as well as several pre-clinical and clinical articles on stannsoporfin.
- Supervised a team of 3 CRAs with liaison with an external CRO and numerous pre-clinical laboratories.
- Developed an extensive investigator network with excellent rapport with key opinion leaders in the field.
- Created a study newsletter that allows all study sites to view key parameters of enrollment, study progress, and monitoring so that the studies are handles according to Good Clinical Practice. Saved thousands of dollars and time in

communication of important information to study sites.

- Was responsible for the Phase 4 clinical program for phenoxybenzamine. Helped to increase sales by having a scientific re-evaluation and removal of black box warning. Wrote and conducted a clinical trial for its use in prostatic disease. Assisted in the development of new labeling that reduced the severity of the warnings previously issued. Developed a protocol for injectable phenoxybenzamine use in the Norwood Procedure. Assisted in the process of obtaining a patent for injectable phenoxybenzamine.
- Prevented removal of triamterene from the market by the FDA by argument that drug bioequivalence was similar to that found in the combination of triamterene and hydrocholorothiazide that was marketed by a competitor. Wrote and published a pharmacodynamic study that specifically showed the value of use of triamterene in conjunction with furosemide in potassium maintenance. Developed a program with the FDA to create a triamterene furosemide drug combination for use in congestive heart failure.
- Supervised a staff of 3 CRAs and coordinated the data management, statistical analysis and report writing for the clinical and pre-clinical programs.
- Managed the activities of several consultants in pre-clinical, laboratory, and clinical venues.
- Joined the American College of Clinical Pharmacology

1998 Raritan Bay Medical Center Perth Amboy, NJ

Hospital Administration, Manager of Physician Billing

- Created a successful internal physician-billing department for emergency room charges that generated 4 million dollars per year of revenue for the hospital and increase of three million dollars per year.
- Member of the hospital corporate compliance committee
- Was responsible for ICD-9 medical coding of all charts from the Emergency Rooms of 2 hospitals and the CPT charges associated with them.
- Was a member of the corporate compliance team of the hospital and acted as internal chart auditor certifying charts for insurance payments
- Supervised a staff of 2 billers, one bookkeeper and one secretary
- Joined the American Society of Clinical Coders

1997 Distinctive Marketing Montclair, NJ

Director of Medical Market Research

- Conducted pharmaceutical market research in various therapeutic areas through primary physician and patent phone interviews. Wrote the final market research reports for management for clients such as Lilly, Astra-Zeneca, and Wyeth
- Performed focus group management and write-ups for clients such as Schering

BENJAMIN LEVINSON, M.D.
91 DEAD TREE RUN ROAD
BELLE MEAD, NJ 08502
908-359-2536 (CELL 908-910-4815)
blevinson@patmedia.net

-Plough.

- Wrote a company newsletter detailing accomplishments and services of the company on a monthly basis.
- Was chosen employee of the month in Nov of 1998
- Joined the Society of Competitive Intelligence Professionals (SCIP).

1990 Cahill Clinic Newark, NJ

Associate Medical Director

- Practiced Internal and Industrial Medicine in a clinic setting with an average patient load of about 50 patients per day.
- Represented the clinic to clients who had contracted for health services with the clinic.

1989 Anaquest Pharmaceutical Milburn , NJ

Senior Associate Clinical Research Director

- Responsible for the Phase 1-3 clinical development for nalmefene, an opioid antagonist with a long-duration of activity. Wrote several studies, conducted investigator meetings and provided medical monitoring for the program. Interacted extensively with the FDA.
- Responsible for the Phase 1-3 development of clebopride to reduce post-operative nausea.
- Supervised a staff of 2 CRAs and 1 data manager.
- Acted as liaison with CRO

1987 E.R. Squibb and Sons, Inc. Princeton, NJ

Associate Clinical Research Director

- Responsible for the Phase 3 clinical development of fosinopril (Monopril). Participated in the protocol writing, investigator site management, medical monitoring, and study budget and contract management. Participated in the corporate project team and developed the investigator publications program for the drug.
- Performed a multi-center study of captopril in combination with hydrocholorothiazide to expand its use beyond present label requirements.
- Chaired the hypertension department committee to evaluate new and novel anti-hypertensives
- Was assistant clinical professor of medicine in the department of hypertension at Robert wood Johnson Medical School

1983 E.R. Squibb and Sons, Inc. Princeton, NJ

Associate/Assistant Clinical Pharmacology Director

BENJAMIN LEVINSON, M.D.
91 DEAD TREE RUN ROAD
BELLE MEAD, NJ 08502
908-359-2536 (CELL 908-910-4815)
blevinson@patmedia.net

- Responsible for the Phase 1 clinical development of aztreonam and new oral monobactam candidates. Participated in the protocol writing, investigator site management, medical monitoring, study budget and contract management. Analyzed the data and wrote the final pharmacokinetic reports to the FDA.
- Performed several Phase 1 studies on new angiotensin-converting enzyme inhibitors such as fosinopril and zofenopril.
- Performed bioequivalence and pharmacokinetic interaction studies on several marketed compounds such as captorpil and fluphenazine.
- Published from the final report data.

EDUCATION

1980 Rutgers University Piscataway, NJ
- M.D.
- Began at Universidad de Guadalajara (1974) transferred to Rutgers in 1978

1974 Rutgers University Newark, NJ
- B.A., Zoology and Physiology
- Graduated Cum Laude.

TRAINING

1983 Robert Wood Johnson Hospital New Brunswick, NJ

- Completed a Residency Program in Internal Medicine was certified in 1983

PATENTS/PUBLICATIONS

PATENTS

US PATENT # 6,818,763 Issued November, 2004
Preparation of metal mesoporphyrin halide compounds

PUBLICATIONS

Applied Clinical Trials. 2005 Jun
Example of a CRO-Industry Partnership
Levinson, B and Mitchel, J.

Int J Clin Pharmacol Ther. 2005 Feb;43(2):92-100.
Attenuation of the kaluretic properties of furosemide by triamterene (Dyrenium) in healthy volunteers.
Levinson B, Shenouda M, Stypinski D.

Clinical Pharmacology and Therapeutics - Vol. 77 Núm. 2
The rationale for the use of an internet-based clinical trial to obtain safety, pharmacokinetic and pharmacodynamic data in a dose-escalation study in hyperbilirubinemia

BENJAMIN LEVINSON, M.D.
91 DEAD TREE RUN ROAD
BELLE MEAD, NJ 08502
908-359-2536 (CELL 908-910-4815)
blevinson@patmedia.net

Mitchel, J.; Levinson, B.

Antimicrob Agents Chemother. 1986 Jun;29(6):1101-3.
Aztreonam concentration in abdominal tissues and bile.
Condon RE, Friedhoff LT, Edmiston CE, Levinson B.

Lancet. 1982 Dec 25;2(8313):1452-3.
The use of smell in differential diagnosis.
Smith M, Smith LG, Levinson B.

J. Hypertension. 1989. Vol. 2. P. 8. (Abstract)
Age is not reason for dose adjustment for fosinopril in hypertension
Levinson, B. and Graney W.F., De Vault A.R. et al

J. Clin. Pharmacology. 1986. Vol. 26. P. 541. (Abstract)
Advanced age per se has no influence on the kinetics of the active diacid of fosinopril
Levinson B., Sugerman A.A., Couchman T. et al.

J Clin Pharmacol (1985) 25, 460. (Abstract)
Lack of kinetic interaction of captopril (CP) and procainamide (PA) in healthy subjects.
Levinson B, Sugerman AA, McKown J.

J.of Clin. Pharm. 2004 Oct (Abstract)
A Study to Evaluate the Dose-Proportionate Pharmacokinetics of Stannsoporfin in Healthy Volunteers
Levinson B, and Shenouda, M

Society Memberships (Inactive)

American College of Clinical Pharmacology
American Society of Professional Coders
Society for Competitive Intelligence Professionals

Articles

Dietary supplementation with n-3 polyunsaturated fatty acids and vitamin E after myocardial infarction: results of the GISSI-Prevenzione trial

*GISSI-Prevenzione Investigators** (Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico)

Summary

Background There is conflicting evidence on the benefits of foods rich in vitamin E (α-tocopherol), n-3 polyunsaturated fatty acids (PUFA), and their pharmacological substitutes. We investigated the effects of these substances as supplements in patients who had myocardial infarction.

Methods From October, 1993, to September, 1995, 11 324 patients surviving recent (≤3 months) myocardial infarction were randomly assigned supplements of n-3 PUFA (1 g daily, n=2836), vitamin E (300 mg daily, n=2830), both (n=2830), or none (control, n=2828) for 3·5 years. The primary combined efficacy endpoint was death, non-fatal myocardial infarction, and stroke. Intention-to-treat analyses were done according to a factorial design (two-way) and by treatment group (four-way).

Findings Treatment with n-3 PUFA, but not vitamin E, significantly lowered the risk of the primary endpoint (relative risk decrease 10% [95% CI 1–18] by two-way analysis, 15% [2–26] by four-way analysis). Benefit was attributable to a decrease in the risk of death (14% [3–24] two-way, 20% [6–33] four-way) and cardiovascular death (17% [3–29] two-way, 30% [13–44] four-way). The effect of the combined treatment was similar to that for n-3 PUFA for the primary endpoint (14% [1–26]) and for fatal events (20% [5–33]).

Interpretation Dietary supplementation with n-3 PUFA led to a clinically important and statistically significant benefit. Vitamin E had no benefit. Its effects on fatal cardiovascular events require further exploration.

Lancet 1999; **354**: 447–55
See Commentary page ???

Introduction

The protective effects of foods rich in n-3 polyunsaturated fatty acids (PUFA) derived from marine vertebrates, vitamin E (α-tocopherol), and their pharmacological equivalents on cardiovascular risk has been of interest for the past 20 years.[1–4] Since a low rate of coronary heart disease was reported in the Eskimo population exposed to a diet rich in fish oil,[5] several studies have explored and supported antiatherogenic, antithrombotic, and antiarrhythmic effects of n-3 PUFA.[3,4] Although no consensus existed on the underlying mechanism of action, focus was placed on the ability of triglycerides to lower high-dose n-3 PUFA (registration approval was given for this indication), and to modify membrane composition.[2–4] A protective role in the secondary prevention of coronary heart disease was seen for fatty fish in the Diet And Reinfarction Trial (DART).[6]

By contrast, large observational cohort studies[7–10] support the role of vitamin E as an antioxidant against the proatherogenic and prothrombotic effects of LDL oxidation.[11–13] However, controlled trials testing this hypothesis in populations with different background cardiovascular risk produced controversial results. No decrease in cardiovascular events was seen with low-dose (50 mg daily) vitamin E supplementation in smokers;[14] a significant decrease in non-fatal myocardial infarction and an increase in fatal cardiovascular events was reported with a daily regimen of 400–800 mg vitamin E in patients with angiographically proven coronary atherosclerosis.[15]

A possible complementary role for these two dietary components has been purported: vitamin E could improve the role of n-3 PUFA through protection from lipid peroxidation, by acting independently on the same or closely related atherogenic and thrombotic mechanisms, or both.[4,16]

We investigated in the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI)-Prevenzione trial the independent and combined effects of n-3 PUFA and vitamin E on morbidity and mortality after myocardial infarction.[17]

Patients and methods

Patients

We enrolled patients with recent (≤3 months) myocardial infarction. Eligible patients had no contraindications to the dietary supplements (ie, known allergy to n-3 PUFA or α-tocopherol, or known congenital defects of coagulation), were able to provide informed written consent, and had no unfavourable short-term outlook (eg, overt congestive heart failure, cancers, &c). We did not define age limits.

*Investigators listed at end of paper.

Correspondence to: Dr Roberto Marchioli, GISSI-Prevenzione Coordinating Centre, Consorzio Mario Negri Sud, Via Nazionale, 66030 Santa Maria Imbaro, Italy

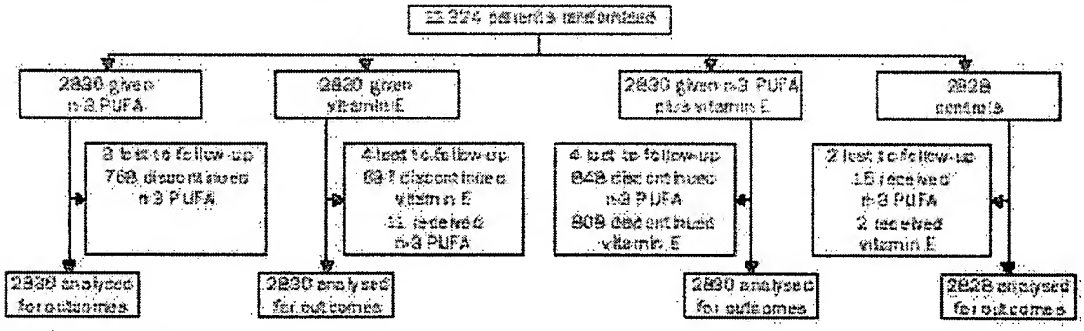

Figure 1: **Trial profile**

Study design

We used a multicentre, open-label design, in which patients were randomly allocated to four treatment groups. In the absence of evidence for preferred doses of treatments, we decided on the daily doses of n-3 PUFA as 1 gelatin capsule containing 850–882 mg eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) as ethyl esters in the average ratio of EPA/DHA 1:2, and 300 mg vitamin E, given as one capsule of synthetic α-tocopherol; these doses used existing available formulations to help compliance in patients already receiving many other long-term treatments. We asked patients to adhere to recommended preventive treatments—aspirin, β-blockers, and inhibitors of angiotensin-converting enzyme (statins were not supported by definitive data on efficacy when the trial was started).

	n-3 PUFA (n=2835)	Vitamin E (n=2830)	n-3 PUFA plus vitamin E (n=2830)	Control (n=2828)	All (n=11 324)
Male/female	2403 (84·7%)/433 (15·3%)	2398 (84·7%)/432 (15·3%)	2451 (86·6%)/379 (13·3%)	2407 (85·1%)/421 (14·9%)	9659 (85·3%)/1665 (14·7%)
Age (years)					
≤50	592 (20·8%)	560 (19·8%)	598 (21·0%)	577 (20·4%)	2325 (20·5%)
51–60	827 (29·1%)	849 (30·0%)	875 (31·0%)	844 (31·0%)	3395 (30·0%)
61–70	943 (33·2%)	946 (33·4%)	930 (32·8%)	937 (33·1%)	3756 (33·1%)
71–80	415 (14·6%)	424 (15·0%)	370 (13·0%)	418 (14·7%)	1627 (14·3%)
>80	59 (2·0%)	51 (1·8%)	59 (2·0%)	52 (1·8%)	221 (1·9%)
Time from AMI to randomisation (days)					
<10	752 (26·5%)	727 (25·7%)	731 (25·8%)	754 (26·7%)	2964 (26·2%)
10–15	641 (22·6%)	661 (23·4%)	665 (23·5%)	637 (22·5%)	2604 (23·0%)
16–30	613 (21·6%)	644 (22·9%)	675 (23·9%)	645 (22·8%)	2577 (22·8%)
≥31	830 (29·3%)	798 (28·2%)	759 (26·8%)	792 (28·0%)	3179 (28·1%)
Secondary diagnoses					
Arterial hypertension	1019 (36·0%)	1007 (35·6%)	1033 (36·5%)	967 (34·2%)	4026 (35·6%)
Diabetes mellitus	405 (14·2%)	426 (15·0%)	426 (15·0%)	426 (15·0%)	1683 (14·8%)
Non-smokers	632 (22·4%)	636 (22·5%)	618 (22·0%)	613 (21·9%)	2499 (22·2%)
Ex-smokers	998 (35·4%)	1016 (36·1%)	972 (34·5%)	953 (34·0%)	3937 (35·0%)
Smokers	1189 (42·2%)	1161 (41·3%)	1223 (43·5%)	1234 (44·0%)	4807 (42·4%)
Body-mass index >30 kg/m²	419 (14·7%)	403 (14·2%)	432 (15·2%)	390 (13·8%)	1644 (14·5%)
Previous myocardial infarction	328 (11·6%)	333 (11·8%)	365 (13·0%)	333 (11·9%)	1357 (12·0%)
Claudication	127 (4·5%)	125 (4·4%)	122 (4·3%)	127 (4·5%)	501 (4·4%)
Angina grade (CCVS)					
No angina	1688 (59·5%)	1667 (59·9%)	1679 (59·3%)	1696 (60·0%)	6730 (59·4%)
No limitation (I)	897 (31·6%)	923 (32·6%)	881 (31·1%)	1095 (31·7%)	3596 (31·8%)
Slight limitation (II)	126 (4·4%)	125 (4·4%)	136 (4·1%)	122 (4·3%)	508 (4·5%)
Severe limitation (III)/at rest (IV)	29 (2·1%)	50 (1·8%)	54 (1·9%)	46 (1·6%)	209 (1·8%)
Dyspnoea grade (NYHA)					
No dyspnoea	968 (34·4%)	941 (33·5%)	955 (34·0%)	966 (34·6%)	3830 (34·1%)
No limitation (I)	1561 (55·0%)	1600 (56·5%)	1554 (54·9%)	1537 (54·3%)	6252 (55·2%)
Dyspnoea on normal/mild exertion (II–III)	287 (10·1%)	264 (9·3%)	294 (10·4%)	201 (10·3%)	1136 (10·0%)
Ejection fraction					
≤0·30	56 (2·3%)	69 (2·9%)	59 (2·5%)	65 (2·7%)	249 (2·6%)
0·31–0·40	283 (11·7%)	245 (10·2%)	279 (11·6%)	264 (11·0%)	1071 (11·1%)
>0·40	2089 (86·0%)	2092 (87·0%)	2059 (85·9%)	2078 (86·3%)	8319 (86·3%)
Premature ventricular beats >10/h	259 (13·1%)	252 (12·6%)	278 (14·1%)	279 (14·1%)	1068 (13·5%)
Previous sustained ventricular tachycardia	17 (0·9%)	25 (1·3%)	18 (0·9%)	13 (0·7%)	73 (0·9%)
Ventricular arrhythmias	373 (18·8%)	376 (18·7%)	400 (20·2%)	385 (19·4%)	1534 (19·3%)
Positive exercise-stress test	550 (29·8%)	511 (27·6%)	542 (29·0%)	534 (29·0%)	2137 (28·9%)
Mean (SD) characteristics					
Age	59·4 (10·7%)	59·5 (10·5%)	59·1 (10·5%)	59·4 (10·5%)	59·4 (10·6%)
Days since diagnosis of AMI	25·4 (21·0%)	25·0 (20·7%)	24·7 (20·7%)	25·2 (21·1%)	25·1 (20·1%)
Body-mass index (kg/m²)	26·5 (3·9%)	26·6 (3·6%)	26·6 (3·6%)	26·4 (3·5%)	26·5 (3·7%)
Ejection fraction	52·6 (10·6%)	52·9 (10·5%)	52·4 (10·5%)	52·5 (10·8%)	52·6 (10·6%)
Lipids (mg/dL)					
Total blood cholesterol	210·2 (42·1%)	211·1 (42·4%)	210·6 (41·5%)	211·6 (42·3%)	210·9 (42·1%)
LDL cholesterol	137·3 (39·1%)	138·0 (39·1%)	138·2 (38·1%)	138·5 (37·6%)	137·4 (38·0%)
HDL cholesterol	41·5 (11·3%)	41·3 (11·2%)	41·6 (11·5%)	41·7 (12·0%)	41·5 (11·5%)
Triglycerides	162·6 (81·7%)	163·3 (84·3%)	160·3 (80·3%)	161·9 (84·6%)	162·1 (85·6%)

AMI=acute myocardial infarction; CCVS=Canadian Cardiovascular Society; NYHA=New York Heart Association. In some sections numbers do not add up because of missing values.

Table 1: **Baseline characteristics of randomised patients**

	n-3 PUFA (n=2836)	Vitamin E (n=2830)	n-3 PUFA plus vitamin E (n=2830)	Control (n=2828)	All (n=11 324)
Dietary habits					
Fish (≥1 serving/week)					
Baseline	2050 (72·9%)	2053 (73·1%)	2057 (73·3%)	2053 (73·4%)	8213 (73·2%)
6 months	2170 (85·9%)	2184 (87·7%)	2137 (86·2%)	2125 (85·5%)	8616 (86·3%)
42 months	1676 (87·7%)	1622 (87·5%)	1691 (88·1%)	1578 (87·2%)	6527 (87·6%)
Fruit (≥1 serving/day)					
Baseline	2243 (79·9%)	2269 (80·8%)	2239 (79·8%)	2259 (80·9%)	9010 (80·3%)
6 months	2185 (86·7%)	2169 (87·4%)	2191 (88·4%)	2145 (86·7%)	8680 (87·3%)
42 months	1670 (87·9%)	1625 (88·0%)	1635 (87·5%)	1590 (88·5%)	6520 (88·0%)
Fresh vegetables (≥1 serving/day)					
Baseline	1121 (39·8%)	1088 (38·7%)	1145 (40·8%)	1107 (39·6%)	4461 (39·7%)
6 months	1341 (53·0%)	1299 (52·1%)	1333 (53·8%)	1331 (53·4%)	5304 (53·1%)
42 months	1055 (55·1%)	1010 (54·4%)	1026 (54·6%)	988 (54·4%)	4079 (54·6%)
Olive oil (regularly)					
Baseline	2092 (74·3%)	2085 (74·3%)	2016 (71·8%)	2066 (73·9%)	8259 (73·6%)
6 months	1998 (79·1%)	1993 (80·2%)	1955 (79·0%)	1990 (80·0%)	7935 (79·6%)
42 months	1566 (82·2%)	1542 (83·4%)	1542 (82·5%)	1486 (82·0%)	6136 (82·5%)
Pharmacological therapy					
Antiplatelet drugs					
Baseline	2601 (92·2%)	2565 (91·2%)	2582 (91·8%)	2562 (91·5%)	10 310 (91·7%)
6 months	2308 (88·2%)	2262 (87·4%)	2261 (87·5%)	2267 (88·3%)	9098 (87·8%)
42 months	1707 (83·4%)	1649 (82·5%)	1685 (83·2%)	1627 (82·1%)	6668 (82·8%)
Angiotensin-converting-enzyme inhibitors					
Baseline	1298 (46·0%)	1287 (45·7%)	1352 (48·1%)	1343 (48·0%)	5280 (46·9%)
6 months	1033 (39·5%)	1074 (41·5%)	1045 (40·4%)	1083 (42·2%)	4235 (40·9%)
42 months	788 (38·5%)	774 (38·7%)	826 (40·8%)	754 (39·0%)	3142 (39·0%)
β-blockers					
Baseline	1237 (43·9%)	1261 (44·8%)	1250 (44·4%)	1238 (44·2%)	4986 (44·3%)
6 months	1092 (41·7%)	1085 (41·9%)	1052 (40·7%)	1043 (40·6%)	4272 (41·2%)
42 months	807 (39·4%)	790 (39·5%)	764 (37·7%)	738 (37·2%)	3099 (38·5%)
Cholesterol-lowering drugs					
Baseline	124 (4·4%)	130 (4·6%)	135 (4·8%)	145 (5·1%)	534 (4·7%)
6 months	782 (28·6%)	780 (28·8%)	757 (27·9%)	786 (29·1%)	3105 (28·6%)
42 months	1003 (46·0%)	962 (44·8%)	1013 (46·7%)	941 (44·4%)	3919 (45·5%)
Revascularisation procedures*					
CABG or PTCA					
Baseline	135 (4·8%)	142 (5·0%)	157 (5·6%)	126 (4·5%)	560 (5·0%)
6 months	433 (15·3%)	439 (15·5%)	481 (17·0%)	429 (15·2%)	1782 (15·7%)
42 months	689 (24·3%)	651 (23·0%)	707 (25·0%)	670 (23·7%)	2717 (24·0%)

CABG=coronary artery bypass; PTCA=percutaneous transluminal coronary angioplasty.
In some sections numbers do not add up because of missing values. Patients alive at baseline=11 324; 6 months=11 092; and 42 months=8259.
*Number and percentage of patients revascularised during study are cumulative.

Table 2: **Dietary habits and main therapeutic interventions at baseline and during study**

Patients were randomly assigned n-3 PUFA alone (n=2836), vitamin E alone (n=2830), n-3 PUFA and vitamin E combined (n=2830), or no supplement (control, n=2828). Treatment was administered by investigators or, in some instances, by hospital pharmacists.

Randomisation was done over the telephone and by computer network. Treatments were automatically assigned from a program based on the biased-coin algorithm, which allowed stratification by hospital.[9] Randomisation data were kept at the coordinating centre.

We planned the procedures of the trial to mimic as far as possible the routine of care after myocardial infarction. We scheduled follow-up visits at 6 months, 12 months, 18 months, 30 months, and 42 months that included clinical assessment and the administration of a food-frequency questionnaire. We measured compliance by refilling drug supplies every 3 months. Blood samples were taken for measurement of lipids at baseline and at follow-up visits for a companion study run by the research group of the Italian Society of Clinical Biochemistry (SIBioC) that was investigating the quality control and the monitoring of main biochemical markers.[10]

The primary combined efficacy endpoints were: the cumulative rate of all-cause death, non-fatal myocardial infarction, and non-fatal stroke; and the cumulative rate of cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke. We did secondary analyses for each component of the primary endpoints, and for the main causes of death.

Myocardial infarction was taken to be present if the investigator had identified this complication on a standard form or if a death certificate or hospital records showed a fatal myocardial infarction. Non-fatal acute myocardial infarction was defined as at least two of the following: chest pain of typical intensity and duration; ST segment elevation or depression of 1 mm or more in any limb lead of the electrocardiogram, of 2 mm or more in any precordial lead, or both; or at least a doubling in necrosis enzymes. Diagnosis of non-fatal stroke required unequivocal signs or symptoms of remaining neurological deficit, with sudden onset and a duration of more than 24 h. Diagnosis of fatal stroke also used these criteria. Alternatively, we used the diagnosis documented in hospital records or on death certificates. The validation of the clinical events included in the primary endpoints was assured by an ad-hoc committee of expert cardiologists and neurologists blinded to patients' treatment assignment.

The study was conceived, managed, and analysed by the coordinating centre, under the responsibility of the steering committee. We obtained the approval of existing ethics committees before the start of the trial. All patients gave informed written consent. The external safety and efficacy monitoring committee did one interim analysis, masked to treatment assignment.

Statistical methods

We estimated that the cumulative rate of death, non-fatal myocardial, and stroke in the control group over the planned 3·5 years of the study would be 20%. The sample size of the trial was calculated to compare the rate of the main endpoint in each of the three study-drug groups to that of the control group (3000 patients per group, relative-risk decrease 20%) and to test the hypothesis that the combined treatment would decrease by a further 20% the rate of the main endpoint compared with n-3 PUFA alone or vitamin E alone. According to the protocol,

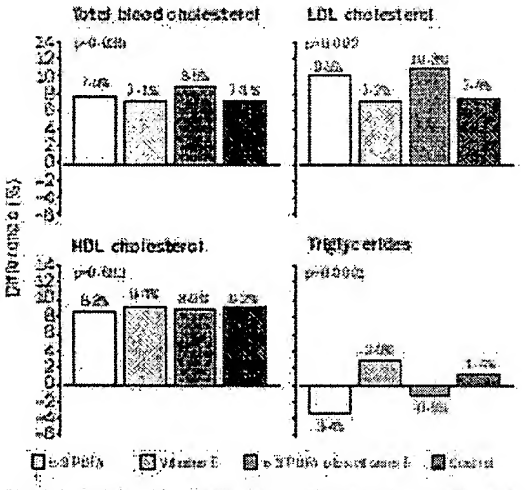

Figure 2: **Percentage differences in blood lipid concentrations at 6 months**

Bars show percentage change from baseline to 6 months.

follow-up data were right-censored at 42 months, when follow-up information on the vital status of patients, through clinical visits or census, was available for 99·9% of the population. Analysis was done by intention to treat and according to the two strategies defined in the protocol: first, a factorial design with two-way analysis of efficacy of n-3 PUFA supplements compared with no n-3 PUFA and efficacy of vitamin E supplements compared with no vitamin E; second, a four-way analysis of efficacy of n-3 PUFA supplements, vitamin E supplements, and the combined treatment compared with control, as well as the efficacy of the combined treatment compared with individual interventions.[9,21]

We analysed data by Kaplan-Meier survival curves and the log-rank test. Treatment efficacy was assessed by baseline values of the risk-stratification variables fitting various Cox's regression models adjusted for the confounding effect of relevant prognostic indicators. The assumption of proportionality in the hazard functions for the experimental groups was assessed visually.[22] In addition, we fitted a logistic function to the data, which gave the same results as the fitting of the Cox's proportional hazards model. Criteria for hierarchical use of events as endpoints have been reported elsewhere.[23] Briefly, we first looked at information on vital status and, if the patient was alive at the end of the study, we assessed whether a non-fatal event had occurred. We used the Kruskal-Wallis test for continuous variables. All p values are two-sided.

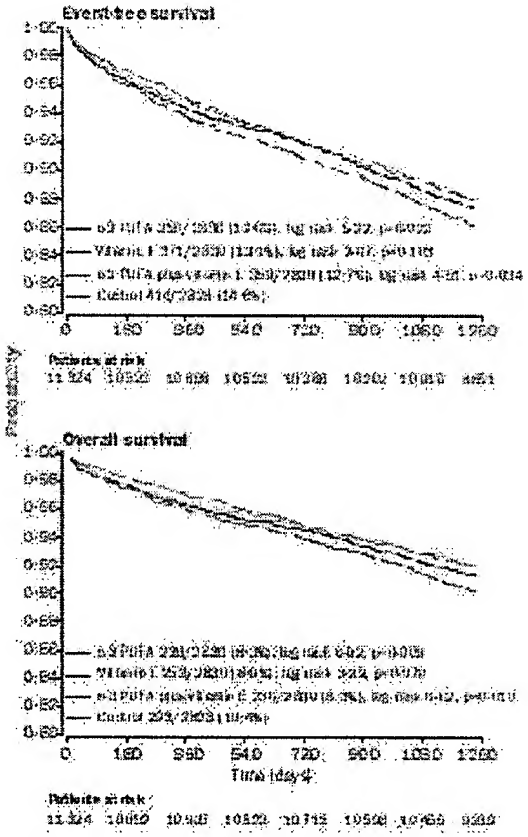

Figure 3: **Event-free survival and overall survival**

To explore interaction, we fitted multivariate models including the two experimental treatments and the interaction term. If significant, the latter indicates effect modification when the two treatments are given together.

Results

Between October, 1993, and September, 1995, 11 324 patients were recruited (figure 1) by 172 participating centres (130 cardiological departments and 42 rehabilitation centres) across Italy. Information on vital status at the end of the study was 99·9% complete for a

	All (n=11 324)	Two-way analysis: n-3 PUFA (n=5669)	Two-way analysis: Control (n=5668)	Two-way analysis: Relative risk (95% CI)	Four-way analysis: n-3 PUFA (n=2536)	Four-way analysis: Control (n=2828)	Four-way analysis: Relative risk (95% CI)
Main endpoints							
Death, non-fatal MI, and non-fatal stroke	1500 (13·3%)	715 (12·6%)	785 (13·9%)	0·90 (0·82–0·99)	350 (12·3%)	414 (14·6%)	0·85 (0·74–0·98)
Cardiovascular death, non-fatal MI, and non-fatal stroke	1155 (10·2%)	547 (9·7%)	608 (10·8%)	0·89 (0·80–1·01)	262 (9·2%)	322 (11·4%)	0·80 (0·68–0·95)
Secondary analyses							
All fatal events	1017 (9·0%)	472 (8·3%)	545 (9·6%)	0·86 (0·76–0·97)	236 (8·3%)	293 (10·4%)	0·80 (0·67–0·94)
Cardiovascular deaths	639 (5·6%)	291 (5·1%)	348 (6·2%)	0·83 (0·71–0·97)	136 (4·8%)	193 (6·8%)	0·70 (0·56–0·87)
Cardiac death	520 (4·6%)	228 (4·0%)	292 (5·2%)	0·78 (0·65–0·92)	108 (3·8%)	165 (5·8%)	0·65 (0·51–0·82)
Coronary death	479 (4·2%)	214 (3·8%)	265 (4·7%)	0·80 (0·67–0·95)	100 (3·5%)	151 (5·3%)	0·65 (0·51–0·84)
Sudden death	236 (2·5%)	122 (2·2%)	164 (2·9%)	0·74 (0·58–0·93)	55 (1·9%)	99 (3·5%)	0·55 (0·40–0·76)
Other deaths	378 (3·3%)	181 (3·2%)	197 (3·5%)	0·91 (0·74–1·11)	100 (3·5%)	100 (3·5%)	0·99 (0·75–1·30)
Non-fatal cardiovascular events	578 (5·1%)	287 (5·1%)	291 (5·1%)	0·98 (0·83–1·15)	140 (4·9%)	144 (5·1%)	0·96 (0·76–1·21)
Other analyses							
CHD death and non-fatal MI	909 (8·0%)	424 (7·5%)	485 (8·6%)	0·87 (0·76–0·99)	196 (6·9%)	259 (9·2%)	0·75 (0·62–0·90)
Fatal and non-fatal stroke	178 (1·6%)	98 (1·7%)	80 (1·4%)	1·21 (0·91–1·63)	54 (1·9%)	41 (1·5%)	1·30 (0·87–1·96)

MI=myocardial infarction; CHD=coronary heart disease.
Patients with two or more events of different types appear more than once in columns but only once in rows.

Table 3: **Overall efficacy profile of n-3 PUFA treatment**

	All (n=11 324)	Two-way analysis			Four-way analysis		
		Vitamin E (n=5666)	Control (n=5668)	Relative risk (95% CI)	Vitamin E (n=2830)	Control (n=2828)	Relative risk (95% CI)
Combined endpoints							
Death, non-fatal MI, and non-fatal stroke	1500 (13·3%)	730 (12·9%)	770 (13·6%)	0·95 (0·86–1·05)	371 (13·1%)	414 (14·6%)	0·89 (0·77–1·03)
Cardiovascular death, non-fatal MI, and non-fatal stroke	1155 (10·2%)	571 (10·1%)	584 (10·3%)	0·98 (0·87–1·10)	285 (10·1%)	322 (11·4%)	0·88 (0·75–1·04)
Secondary analyses							
All fatal events	1017 (9·0%)	488 (8·6%)	529 (9·3%)	0·92 (0·82–1·04)	252 (8·9%)	293 (10·4%)	0·86 (0·72–1·02)
Cardiovascular deaths	639 (5·6%)	310 (5·5%)	329 (5·8%)	0·94 (0·81–1·10)	155 (5·5%)	193 (6·8%)	0·80 (0·65–0·99)
Cardiac death	520 (4·6%)	247 (4·4%)	273 (4·8%)	0·91 (0·76–1·08)	127 (4·5%)	165 (5·8%)	0·77 (0·61–0·97)
Coronary death	479 (4·2%)	228 (4·0%)	251 (4·4%)	0·91 (0·76–1·09)	114 (4·0%)	151 (5·3%)	0·75 (0·59–0·96)
Sudden death	286 (2·5%)	132 (2·3%)	154 (2·7%)	0·86 (0·68–1·08)	65 (2·3%)	99 (3·5%)	0·65 (0·48–0·89)
Other deaths	378 (3·3%)	178 (3·1%)	200 (3·5%)	0·89 (0·73–1·09)	97 (3·4%)	100 (3·5%)	0·96 (0·73–1·28)
Non-fatal cardiovascular events	578 (5·1%)	294 (5·2%)	284 (5·0%)	1·04 (0·88–1·22)	147 (5·2%)	144 (5·1%)	1·02 (0·81–1·28)
Other analyses							
CHD death and non-fatal MI	909 (8·0%)	454 (8·0%)	455 (8·0%)	1·00 (0·88–1·14)	226 (8·0%)	259 (9·2%)	0·87 (0·73–1·04)
Fatal and non-fatal stroke	178 (1·6%)	83 (1·5%)	95 (1·7%)	0·87 (0·65–1·17)	39 (1·4%)	41 (1·5%)	0·95 (0·61–1·47)

MI=myocardial infarction; CHD=coronary heart disease.
Patients with two or more events of different types appear more than once in columns but only once in rows.

Table 4: **Overall efficacy profile of vitamin E treatment**

total person-time of 38 053 years. Median time from the index myocardial infarction to randomisation was 16 days. Baseline demographic and clinical characteristics were well balanced across the groups (table 1) and define a relatively low-risk population, with 16% of patients aged 70 years or older, 14% with an echo-documented ejection fraction of 40% or less, and 29% with positive exercise-stress tests. Dietary habits, recommended secondary-prevention treatments, and revascularisation procedures at baseline and during the study were also well balanced across all groups (table 2).

Compared with baseline values, there were no clinically important changes for cholesterol (total, HDL, and LDL), glycaemia, and fibrinogen in any of the treatment groups at the first visit (figure 2). The difference in blood lipids, however, was more slight than any other value during the study (data not shown). Compared with controls, the small decrease in triglyceride concentrations was significant in patients receiving n-3 PUFA.

The full profile of the effects of n-3 PUFA is summarised in table 3. In the two-way factorial analysis, the 10% relative decrease in risk for the combined primary endpoint of death, non-fatal myocardial infarction, and non-fatal stroke was significant (95% CI 1–18, p=0·048), but the decrease in risk for the other combined endpoint of cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke was not significant (11% [1–20], p=0·053).

The four-way analysis provides a clearer profile of the effects of n-3 PUFA (figure 3), with a relative decrease in risk for the combined endpoint of 15% (2–26, p=0·023) and for cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke of 20% (5–32, p=0·008).

Analyses of the individual components of the main endpoint showed that the decrease in mortality (20% for total deaths, 30% for cardiovascular deaths, and 45% for sudden deaths) which was obtained with n-3 PUFA accounted for all the benefit seen in the combined endpoint. There was no difference across the treatment groups for non-fatal cardiovascular events. The results of the tests for interaction were not significant when the two combined endpoints and overall mortality were analysed. The significance values reached when a similar analysis was applied to the individual components of the endpoints (p=0·0242 for cardiovascular mortality; p=0·0226 for coronary mortality; p=0·024 for fatal plus non-fatal coronary events; and p=0·010 for sudden death) better approximate the true unconfounded effect of n-3 PUFA and show that the results of the four-way analysis are not influenced by an effect modification due to the combination of the two treatments.

Patients receiving vitamin E and controls did not differ significantly when data were analysed according to the factorial design (table 4). The lack of evidence of effect is similar for the combined endpoint and for its individual components. The results were similar for the combined endpoints and overall mortality analysed by treatment group. An indication of a possible beneficial effect of vitamin E is provided, however, in the secondary analyses of the individual components of cardiovascular death of the combined endpoints, for which the increasing benefit (from 20% for all cardiovascular deaths, to 35% for sudden death) is similar to the picture for n-3 PUFA. The absence of a difference in the rate of non-fatal cardiovascular events between vitamin E and the control group is also similar to the findings related to n-3 PUFA.

The results for combined treatment compared with controls are shown in table 5. The effects seen on the primary combined endpoint and on total mortality were consistent with those obtained with n-3 PUFA alone. No increased benefit was apparent when the rate of the combined endpoint of death, non-fatal myocardial infarction, and non-fatal stroke that was seen in patients receiving n-3 PUFA plus vitamin E was compared with

	n-3 PUFA plus vitamin E (n=5666)	Control (n=2828)	Relative risk (95% CI)
Main endpoints			
Death, non-fatal MI, and non-fatal stroke	359 (12·7%)	414 (14·6%)	0·86 (0·74–0·99)
Cardiovascular death, non-fatal MI, and non-fatal stroke	285 (10·1%)	322 (11·4%)	0·88 (0·75–1·03)
Secondary analyses			
All fatal events	236 (8·3%)	293 (10·4%)	0·80 (0·67–0·95)
Cardiovascular deaths	155 (5·5%)	193 (6·8%)	0·80 (0·65–0·99)
Cardiac death	120 (4·2%)	165 (5·8%)	0·72 (0·57–0·91)
Coronary death	114 (4·0%)	151 (5·3%)	0·75 (0·59–0·96)
Sudden death	67 (2·4%)	99 (3·5%)	0·67 (0·49–0·92)
Other deaths	81 (2·9%)	100 (3·5%)	0·80 (0·60–1·09)
Non-fatal cardiovascular events	147 (5·2%)	144 (5·1%)	1·01 (0·80–1·27)
Other analyses			
CHD death and non-fatal MI	226 (8·1%)	259 (9·2%)	0·87 (0·73–1·04)
Fatal and non-fatal stroke	44 (1·6%)	41 (1·5%)	1·06 (0·70–1·63)

MI=myocardial infarction; CHD=coronary heart disease.
Patients with two or more events of different types appear more than once in columns but only once in rows.

Table 5: **Overall efficacy profile of n-3 PUFA plus vitamin E treatment**

the group receiving n-3 PUFA alone (1·01 [0·87–1·17) or with patients treated with vitamin E alone (0·96 [0·83–1·12]).

At 1 year and at the end of the study, 11·6% and 28·5% of patients receiving n-3 PUFA and 7·3% and 26·2% of those receiving vitamin E, respectively, had permanently stopped taking the study drug. Conversely, during the whole course of the study, only two patients not assigned vitamin E and 26 patients not assigned n-3 PUFA were receiving these drugs. Side-effects were reported as a reason for discontinuing therapy for 3·8% of patients in the n-3 PUFA groups, and in 2·1% of those in the vitamin E groups. Overall, gastrointestinal disturbances and nausea were the most frequently reported side-effects (4·9% and 1·4% of n-3 PUFA recipients, and 2·9% and 0·4% of vitamin E recipients, respectively).

Cancer occurred in 61 (2·2%) patients in the control group, in 77 (2·7%) in the n-3 PUFA group, in 73 (2·6%) in the vitamin E group, and in 65 (2·3%) in the combined treatment group. There were 33 (1·2%) non-fatal cases of cancer in the control group, 41 (1·5%) in the n-3 PUFA group, 35 (1·2%) in the vitamin E group, and 26 (0·9%) in the combined treatment group.

Discussion

Treatment with n-3 PUFA significantly decreased, over 3·5 years, the rate of death, non-fatal myocardial infarction, and stroke. No effect was seen for vitamin E. When data were analysed by four-way analysis, the size of the beneficial effect of n-3 PUFA became more evident and more clearly significant; the absence of a significant effect was confirmed for vitamin E.

The degree of the effects on rates of death deserves to be specifically highlighted and is suggestive of hypotheses that could have more general implications for secondary-prevention trials in patients who have had myocardial infarction, as well as for pathophysiological interpretation of trial results. The results obtained with n-3 PUFA are consistent with those of the DART trial.[9] They found a 29% decrease over 2 years in overall mortality in men who ate fatty fish twice a week, with no decrease in the rate of non-fatal myocardial infarction. This pattern of effects was reproduced in two large-scale observational studies, the Health Professionals Study[24] and the US Physicians' Health Study.[25] Significant associations between fish intake and lower risk of coronary heart disease were shown in the Zutphen study,[26] the 30-year follow-up of the Western Electric study,[27] the observational cohort of the Multiple Risk Factor Intervention Trial,[28] and the Honolulu Heart Program.[29] The significant results of the Lyon Diet Heart study[30] and of the Indian trial by Singh and colleagues,[31] strongly suggest a protective effect of n-3 PUFA. Because of the high frequency of stroke of non-defined cause, there were only 11 haemorrhagic strokes and, therefore, distribution in the experimental groups could not be clearly inferred.

The pathophysiological basis of the clinical and epidemiological suggestions in favour of a more direct cardiac effect of n-3 PUFA has been explored in a wealth of experimental, animal,[32–35] human,[36–39] and in-vitro[40–48] studies, which together support a role for n-3 PUFA on arrhythmogenesis. The lack of evidence of benefit on atherosclerotic-thrombotic events, despite the well-documented activity of n-3 PUFA on eicosanoid metabolism, inflammation, tissue factor, β-oxidation, endothelial dysfunction, cytokine growth-factors, and gene expression of adhesion molecules, is difficult to explain.[2–4] In our trial, an explanation could partly be the intensive preventive interventions that were documented for the whole duration of the study (table 2).[16]

By contrast with n-3 PUFA, the results for vitamin E did not support the strong epidemiological evidence available at the beginning of the trial and to date,[1,7–13] although the significant decrease of cardiovascular deaths in the four-way analysis cannot be easily dismissed. The information available before the GISSI-Prevenzione trial was contradictory. The suggestion of a striking decrease in non-fatal myocardial infarction, and of a non-significant excess of total and cardiovascular deaths originated from a trial that had severe weaknesses in the methods.[47,48] The data on the absence of any significant effect of low doses (50 mg daily) of vitamin E on cardiovascular death, and on the non-significant (positive and negative) modifications of non-fatal cardiovascular events were obtained in a population that could not be compared with that in our study.[14,49]

Discrepant findings between expectations of benefit based on epidemiological observations and results of clinical trials, however, are not especially surprising.[50,51] The biological background of the suggested mechanisms of action of vitamin E[5,10–13,52] should be considered in the general framework of the biological effects of all the other treatments already prescribed to myocardial-infarction patients, as well as the effects of those attributable to the protection of the Mediterranean eating habits of the GISSI population.[53,54] In addition, it is possible that a longer duration of intervention is needed to allow the action of biological mechanisms of benefit, which might be different from those of n-3 PUFA, and to shift significantly the overall risk profile and, as a consequence, the incidence of fatal events. However, similar considerations would apply also to n-3 PUFA, for which the same experimental context produced consistently positive and significant results.

To better qualify the results of our trial, a few comments are appropriate with respect to the doses of experimental treatments, the open design of the study, the overall clinical importance, and the implications of the size of the effects seen with n-3 PUFA.

The regimen we used for n-3 PUFA corresponds to a diet that contains a large amount of fatty fish, to be maintained every day (eg, 100 g of fatty fish/day), although most of the data available on the mechanisms of this product had been obtained with much higher, purely "pharmacological" doses of n-3 PUFA (≥3–4 g/day). The choice in favour of a regimen more acceptable for long-term treatment seems also to fit well with the favourable clinical and epidemiological "dietary" results, and with emerging suggestions about other mechanisms of action of n-3 PUFA not directly related to a rapid and substantial modification of the saturation ratio of cell membranes.

The dose of vitamin E that we used was in the lower range of those chosen in other continuing clinical trials (only the Alpha-Tocopherol, Beta Carotene Cancer Prevention Study[14] trial used a lower dose of 50 mg/day). It is important to take into account, however, that a dose of 300 mg per day is already in excess of any achievable dose through dietary intake (eg, corresponding to 200 table-spoons of olive oil daily) and is more than ten times higher than current recommended dietary allowances for optimum health in adults. Notably, however, the results of

observational studies suggest no increasing benefit for intakes of vitamin E higher than 100 mg daily.[7,8] In addition, evidence exists that short-term treatment with doses lower than that used in our trial for long-term treatment could prevent LDL oxidation.[55-59] The equivalence between prevention of LDL oxidation and clinical efficacy, however, may be oversimplistic.[34] It is more likely that the gradient of the beneficial effects seen in the most striking results of epidemiological studies reflected the overall behavioural attitudes of the people regularly taking high doses of antioxidant substances over many years. Therefore, the dose of vitamin E that is most effective and safe, as well as the minimum duration of treatment that is required to produce the postulated protective effects of vitamin E are still unknown.[60] Results of continuing large randomised trials with other doses of vitamin E supplements will better elucidate the efficacy profile of this antioxidant substance in lowering cardiovascular risk in patients with myocardial infarction and in other patients, possibly in different clinical settings.[10]

The main risk of any open-label design for a mortality trial could be seen in the possibility of biased behaviour by prescribing doctors and of patients adopting different dietary habits. Our data, however, provide good evidence that dietary habits, secondary prevention with recommended treatments, and revascularisation procedures were well balanced across the four groups throughout the study (table 2). Conversely, the pragmatic strategy used for monitoring was expected to lead to the risk of incomplete compliance, which would have mimicked what is likely to happen in general long-term secondary preventive care in a population whose relatively low-risk profile is already intensively covered with other preventive interventions. The strict adherence to the intention-to-treat principle assures that the effects seen correspond closely to what is achievable in clinical practice.

The size of effect of n-3 PUFA treatment on the primary endpoint of total death, non-fatal myocardial infarction, and non-fatal stroke could be quantified as corresponding to a 10% relative decrease in risk in the two-way analysis and to a 15% relative decrease in risk in the four-way analysis. Although significant, these results are clearly lower than the 20% relative decrease of risk expected in our original planning. An efficacy result that is smaller than expected is quite common in trials in which patients receive more intensive background treatments than populations taken as reference at the time of trial design. Therefore, the rate of events in the control group that was 25% less than expected was not surprising. Although the four-way analysis, which avoids the possible interference of the interaction of effects between treatments, should be seen preferentially as the one showing the "true" results, it is important to take into account that the more relevant effects were seen on the harder component of the primary combined endpoint (20% relative decrease overall and 30% relative decrease of cardiovascular mortality). The effect of multiple comparisons of the various components of the endpoint checked with appropriate statistical approaches did not modify importantly the significance values of the four-way analysis for fatal events.

In this population of patients who had myocardial infarction and Mediterranean dietary habits, and who were well treated with up to date preventive pharmacological interventions, long-term n-3 PUFA 1 g daily, but not vitamin E 300 mg daily, was beneficial for death and for combined death, non-fatal myocardial infarction, and stroke. All the benefit, however, was attributable to the decrease in risk for overall and cardiovascular death.

Investigators of the GISSI-Prevenzione trial

Steering committee—F Valagussa (chairman, Ospedale San Gerardo, Monza), M G Franzosi (Istituto Mario Negri, Milano), E Geraci (Ospedale Cervello, Palermo), N Mininni (Ospedale Monaldi, Napoli), G L Nicolosi (Ospedale Civile, Pordenone), M Santini (Ospedale San Filippo Neri, Roma), L Tavazzi (Policlinico San Matteo, Pavia), C Vecchio (Casa di Cura Montallegro, Genova).

Scientific and organising secretariat—R Marchioli (coordinator), E Bomba, C Chieffo, A P Maggioni, C Schweiger, G Tognoni.

Data management and analysis—F Barzi, A V Flamminio, R M Marfisi, M Olivieri, C Pera, A Polidoro, E Santoro, R Zanu.

Safety and data monitoring committee—L Pagliaro (chairman), E Correale, A Del Favero, U Loi, E Marubini.

Scientific committee—L Campolo, A Casari, G Di Minno, M B Donati, M Galli, M Gattone, S Garattini, M Mancini, P Marino, G M Santoro, C Scardulla, G Specchia.

Event adjudicating committee—A Cericola, D Di Gregorio, R Di Mascio, G Levantesi, L Mantini, G Mastrogiuseppe, C Tucci.

Quality control of laboratory data (Italian Society of Clinical Biochemistry)—P Mocarelli (chairman), R Baldinelli, F Ceriotti, A Colonna, C Cortese, G Fortunato, C Franzini, F Gonano, M S Graziani.

GISSI-Prevenzione trial centres

Alba CN Ospedale S Lazzaro: E Actis, S Castiglione F Marta; Ancona Ospedale INRCA: L Quattrini, E Pactaroni; Aosta Ospedale Regionale: G Begliuomini, V Bigo, Arco TN, Ospedale Provinciale S Pancrazio: C Sarno, D Urbani, R Santoni, Arenzano GE Ospedale La Colletta: R Criffo, Arezzo Ospedale Civile: R Perticucci; Arienzo CE Ospedale S Felice a Cancello: C Medici, C De Matteis, Avellino Ospedale Civile: L Marino, G Stanco, G Rosato, Aversa CE Ospedale G Moscati: P Iodice, G Lucca; Barga LU Ospedale S Francesco: D Bernardi, C Volterrani, L Lotti, A Lanciani; Bari Ospedale Policlinico: I De Luca, Bari Policlinico di Bari: I De Luca, D Traversa, G Castellaneta, Bari-Carbonara BA Ospedale di Venere: N D'Amato, O Pierfelice, Barletta BA Ospedale Umberto I: D Messina, G Sarcina, A Mascolo, C Acquaviva, Battipaglia SA Ospedale Civile: M Maina, G Mondillo, M D Ricrio, Belluno Ospedale Civile: G Cargnel, A Da Roid, Benevento Ospedale G Rummo: V Viola; Bologna Ospedale Bellaria: S Urbinati, P Passarelli, Bosisio Parini LC Ospedale di Bosisio Parini: G Gullace, Bovolone VR Ospedale S Biagio: F Peretto, A Pasini, Brescia Ospedale S Orsola, Fatebenefratelli: G Ghizzoni, Brindisi Ospedale Generale Reg A Di Summa: A Storelli, D Zuffiano, N De Giorgio, C Andriullo, Busto Arsizio VA Ospedale Generale Provinciale: E Cecchetti, A Medici, Cagliari Ospedale SS Trinità: F Scano, W Boi, Camposampiero PD Ospedale Civile: P Turrini, A Zampiero, A Pantaleoni; Casale Monferrato AL Ospedale S Spirito: M T Curti, M Giglio, F Pizzetti; Casarano LE Ospedale F Ferrari: G Pettinati, S Ciricugno, F De Santis; Caserta Ospedale Civile: C Chieffo, C Riccio, A Cardillo, A Palermo; Castellammare di Stabia NA Ospedale S Leonardo: N Di Martino, R Longobardi, E Murena, L De Vivo, Castelnuovo di Garfagnan LU Ospedale Civile: E Nannini, Castrovillari CS Ospedale Civile Ferrari: C Calvelli, F Dulcetti, Catania Ospedale POU S Currò e S Luigi Gonzaga: A Circo, S Raciti, Catania Ospedale Cannizzaro: R Coco, M Franco, A Calassi, Catania Ospedale Garibaldi: S Mangiameli, C Dell'Ali, Catanzaro Ospedale Mater Domini: C Borrello, M L Mazza, P Cirelo, Cecina LI Ospedale di Cecina: F Chiesa, F Mazzinghi, Cernusco sul Naviglio MI Ospedale Civile: A Sagone, C De Ponti, P Barbieri, Chiari BS Presidio Ospedaliero di Chiari: C Bellet, L V Bertoli, E Podavini, Chieti Ospedali Riuniti SS Annunziata: A Rossi, Chivasso TO Ospedale Civico: G Borello, L Garzaro, E Brscic, Città di Castello PG Ospedale Civile: F Giani, M R Pieracci, G Gamberi, Colleferro RM Ospedale Civile: M Mariani, E Venturini, Comacchio FE Ospedale Civile S Camillo: A Mancinelli, P Yannacopulu, Como CO Ospedale S Anna: R Jemoli, F Tettamanti, A Zagami, G Ferrari, Copertino LE Ospedale S Giuseppe da Copertino: G De Rinaldi, Correggio RE Ospedale S Sebastiano: A Barbieri, L Lusetti, Corlona AR Ospedale S Maria della Misericordia: F Cesoni, R Nasorri, M Mallaini, Cosenza Ospedale INRCA: E Feraco, A Lauro, Desio MI Ospedale Civile: P Bertocchi, W Donzelli, G Iacuitti, Domodossola NO Ospedale S Biagio: F Barba, A Alfieri, Eboli SA Centro Ebolitano di Medica Fisica e Riabilitativa: G Parrilli, Erba CO Ospedale Fatebenefratelli: C Falcone, Fasano Del Garda BS Ospedale di Fasano del Garda: B Cerri, Firenze Ospedale

S Giovanni di Dio: S Badolati, M Filice, M Zerauschek. Firenze Osp Careggi: A Santini, P Pucci, E Dovellini. Fivizzano MS Ospedale S Antonio Abate: F Rossi, P Pacetti. Foggia Ospedali Riuniti: D De Matteis. Foligno PG Ospedale Civile: R Liberati, L Meniconi, V Menichini, G Spogli. Forlì Ospedale Morgagni-Pierantoni: R Puggioni, M Balestra. Gallipoli LE Ospedale S Cuore di Gesu': E Cristallo. Garbagnate Milanese MI Ospedale Santa Corona: M T Catanzaro. Gevardo BS Ospedale La Memoria: C Pasini. Genova Ospedale Celesia: A Magioncalda. Genova Sestri Ponente Ospedale Padre Antero Micone: L Moroni, M V Iannetti. Gorizia Ospedale Civile: R Marini, M Spanghero. Grosseto Ospedale Misericordia: A Cresti, N Svetoni. Guastalla RE Ospedale Civile: E Cremaschi. Gussago BS Centro Medico di Gussago: S Scalvini, E Zanelli. Imola BO Ospedale Civile Nuovo: R Leghissa, M De Giovanni. Isernia Ospedale F Veneziale: V Mazza, A Evangelista. La Spezia Ospedale S Andrea: A Motto, G Tonelli. Lanciano CH Ospedale Civile: D Tullio, C Tucci. Lavagna GE Ospedale Civile: A Carfagnini, C Gaggioli. Lodi MI Ospedale Maggiore: M Orlandi. Lucca Centro Medico Alba: A Boni. Magenta MI Ospedale Fornaroli: G Bardelli, G Maggi, A Barosi, A Grittini. Mantova Ospedale Carlo Poma: A Izzo, G Guerra. Matera Ospedali Riuniti: L Veglia. Mazara del Vallo TP Ospedale Abele Ajello: I Fiore. Merate LC Ospedale Civile L Mandic: F Mauri. Mercato S Severino SA Ospedale Civile di Curteri: V Capuano, N Lamaida. Messina Ospedale Piemonte: A M Mangano. Messina Policlinico Universitario: C Maugeri Sacca, A Consolo. Milano Istituto Don Gnocchi: G Mascioccо, A Garascia, B Gara. Milano Ospedale Niguarda Ca' Granda I Divisione De Gasperis: G Cataldo. Milano Ospedale Ca' Granda, Niguarda: A Pezzano. Milano Ospedale Fatebenefratelli: G P Sarina, M Negrini. Milano Ospedale Pio Albergo Trivulzio: S Corallo. Mondovì CN Ospedale Civile: V Parolini, V Lughengo. Monfalcone GO Ospedale Civile: G Zilio, G Gotti. Montecchio Emilia RE Ospedale di Montecchio Emilia: A Navazio. Montescano PV Centro Medico di Riabilitazione: J Assandri, F Cobelli. Monza MI Ospedale Nuovo S Gerardo: F Valagussa, L Sala, E Negro Cusa, A Vincenzi. Mozzo BG Ospedali Riuniti di Bergamo: D Mazzoleni, C Mallinverni, M Doright. Napoli Ospedale Nuovo Cardarelli: N Maurea, A Boccalatte. Napoli Ospedale Nuovo Pellegrini: M Glasl, N Pellegrino, F Ciuccaro. Napoli Ospedale Cardarelli: L Mazzitelli, F R Piantadosi. Napoli Ospedale Loreto Mare: G C Coriglianо, M Accadia, P Cirarini. Napoli Ospedale Monaldi: P Sensale. Napoli Ospedale V Monaldi: N Mininni, R Greco. Novara Ospedale Maggiore: G Cernigliaro. Nuoro Ospedale S Francesco: I Maoddi, C Mureddu, F Soro. Oliveto Citra SA Ospedale di Oliveto Citra: C D'Angelo, P Bottiglieri. Palermo Ospedale B La Ferla Fatebenefratelli: A Castello, G D'Alfonso, N Puraone, L Americo. Palermo Ospedale Cervello: B Di Maria, L Buffa, C Infantolino, A Vivirito. Palermo Ospedale Civico e Benfratelli: U Mirto, G Oliva, C Caruso, D Albanese. Palermo Ospedale Ingrassia: G Barone, F Clemenza, S Cannizzaro, A Giubilato. Palermo Ospedale Villa Sofia: F Mancino, C Mancino, A Battaglia. Passirana di Rho MI Ospedale Civile: C Schweiger, A Frisinghelli, F Rusconi. Pavia Policlinico S Matteo: L Tavazzi. Penne PE Ospedale Civile S Massimo: L Mantini, D Di Gregorio. Perugia Policlinico Monteluce: L Corea, M Cocchieri, M Buccolieri, D Severini. Perugia San Sisto Ospedale R Silvestrini: M Del Pinto, A Notaristefano. Pesaro Ospedale S Salvatore: E Sgarbi, P Cesaroni, P M Baldini. Pescia PT Ospedale Civile: L Incopetti. Piario BG Ospedale A Locatelli: A Locatelli, A Pighini. Piazza Armerina EN Ospedale M Chiello: B Aloisi, G Baiunco. Piedimonte Matese CE Ospedale Civile: A Frasca, P Iuliano, M A Testa. Pinerolo TO Ospedale E Agnelli: E Bellono, M Ferro, R Crivello, M Rizzotti. Piombino LI Ospedale Civile: G Micheli, M Balagi, S Bechi. Piove di Sacco PD Ospedale Immacolata Concezione: C Martines. Pisa Ospedale S Chiara: P Fontanive, E Puccini, E Pardini. Pistoia Ospedali Riuniti: F Del Citerna, E Balli, S Bucci. Pordenone Ospedale S Maria degli Angeli: M Brieda, L Lisotto. Prato Ospedale Civile: L Bini, G Lauri. Putignano BA Ospedale S Michele in Monte Laureto: R Di Martino. Ragusa Ospedale Civile: O Rizza. Ragusa Ospedale MP Arezzo: G Lichtra, R Ferrante. Ravenna Ospedale S Maria delle Croci: G Bellanti. Reggio Calabria Policlinico Madonna della Consolazione: G Pulitano, A Cavalli. Rho MI Ospedale Civile: G De Angelis. Riccione FO Ospedale G Ceccarini: E Fantini, P A Del Corso. Rieti Ospedale Generale Provinciale: S Orazi. Rimini RN Ospedale Civile: M Marzaloni, F Cioppi. Roma Ospedale Addolorata: A De Francesco, C Vartolo, E Severini. Roma Ospedale Fatebenefratelli: F Sgrecia, P Azzolini. Roma Ospedale S Camillo: A Lacché, M Di Segni. Roma Ospedale S Eugenio: F Amaddeo. Roma Ospedale S Pietro FBF: F Ferri, L Panzacchi. Roma Ospedale Santo Spirito: G Greco, G Guaginzzi, F Lumia, L Bordi. Roma Policlinico Casilino: F D'Agostino, P Mancini. S Giovanni Rotondo FG Ospedale Casa Sollievo della Sofferenza: R Fanelli, N Cianfrone, A Villella, E Pisano. S Giovanni Valdarno AR Ospedale Alberti: M Crazzini. S Pellegrino Terme BG Centro di Riabilitazione Clinica Quarenghi: F Burgarella, C Scanzi. Salerno Ospedali Riuniti: A Pipolo, U Bugatti, B Ravera. Saluzzo CN Ospedale Civile: S Reynaud, P Allemano. Sanremo IM Ospedale Civile: L Anselmi. Saronno VA Ospedale Civile: M Croce. Sarzana SP Ospedale S Bartolomeo: R Petacchi, D Bertoli, G Filorizzo. Scandiano RE Ospedale S Magati: G Gambarini, M Zobbi, E Aru. Sciacca AG Ospedale Civile: V Indelicato, A Colletti. Scorrano LE Ospedale Ignazio Veris Delli Ponti: E De Lorenzi, P Marsano. Seriate BG Ospedale Civile: P Giani, T Nicoli, Z Bassani. Sondalo SO Azienda Ospedaliera E Morelli: N Partesana, G Occhi, T Chiodi. Sondrio Ospedale Civile: G Cucchi, S Giustiniani. Spoleto PG Ospedale Civile: L Tramontana, G Maragoni, N Miele. Termoli CB Ospedale Civile S Timoteo: R Pizzicoli, A Cimino, D Staniscia. Torino Ospedale Maria Vittoria: R Trinchero. Torino Ospedale Martini: A Nejrotti, B Zampaglione. Torino Ospedale Molinette: S Bergerone, M Bobbio. Tradate VA Osp Civile: D Barbieri. Trapani Ospedale S Antonio Abate: R Scimeca, M Abrignani, G Braschi. Trebisacce CS Ospedale Civile: L Domiangelo, M Chiatto. Tricase LE Ospedale Provinciale Cardinale Panico: A Galati, P Palma. Urbino PS Ospedale di Urbino: L Miriutiello, G Ghelfi, S Ferri. Vallo Della Lucania SA Ospedale S Luca: A Liguori, G Gregorio, F Catto. Varese Ospedale F del Ponte: G Montemurro, E Rezzonico. Vasto CH Ospedale Civile: G Di Marco, M A Ottaviano, M G Celenza. Verano NO Centro Medico di Riabilitazione: M Gattone, A Mezzani, F Soffiantino. Viterbo Ospedale Belcolle: R Guerra, D Pontillo, E Cicchetti. Vizzolo Predabissi MI Ospedale Predabissi: C Colombo, S Cilloni. Voghera PV Ospedale Civile: P Gandolfi.

Acknowledgments

GISSI is endorsed by Associazione Nazionale Medici Cardiologi Ospedalieri (ANMCO), Firenze, Italy and by Istituto di Ricerche Farmacologiche Mario Negri-Consorzio Mario Negri Sud, Santa Maria Imbaro, Italy.

This study was supported by grants from Bristol-Myers Squibb, Pharmacia-Upjohn, Società Prodotti Antibiotici, and Pfizer. Pharmacia-Upjohn and Società Prodotti Antibiotici supplied marketed capsules containing 850–882 mg EPA/DHA ethyl esters. Vitamin E (acetyl d, l-α-tocopherol) was supplied by Bracco. We thank Miss Barbara Ferri for secretarial help.

References

1 Jha P, Flather M, Lonn E, Farkouh M, Yusuf S. The antioxidant vitamins and cardiovascular disease: a critical review of epidemiological and clinical trial data. *Ann Intern Med* 1995; **123**: 860–72.

2 Simopoulos AP. Omega-3 fatty acids in health and disease and growth and development. *Am J Clin Nutr* 1991; **54**: 438–63.

3 Simopoulos AP. ω-3 fatty acids in the prevention-management of cardiovascular disease. *Can J Physiol Pharmacol* 1997; **75**: 234–39.

4 Marchioli R, Di Pasquale A, per i Ricercatori GISSI-Prevenzione. Il quadro di riferimento biochimico, farmacologico, epidemiologico del GISSI-Prevenzione. *G Ital Cardiol* 1993; **23**: 933–64.

5 Bang HO, Dyerberg J, Hjorne N. The composition of food consumed by Greenland Eskimos. *Acta Med Scand* 1976; **200**: 69–73.

6 Burr ML, Fehily AM, Gilbert JF, et al. Effects of changes in fat, fish, and fibre intakes on death and myocardial reinfarction: diet and reinfarction trial (DART). *Lancet* 1989; **ii**: 757–61.

7 Rimm EB, Stampfer MJ, Ascherio A, Giovannucci E, Colditz GA, Willett WC. Vitamin E consumption and the risk of coronary heart disease in men. *N Engl J Med* 1993; **328**: 1450–56.

8 Stampfer MJ, Hennekens CH, Manson JE, Colditz GA, Rosner B, Willett WC. Vitamin E consumption and the risk of coronary disease in women. *N Engl J Med* 1993; **328**: 1444–49.

9 Kushi LH, Folsom AR, Prineas RJ, et al. Dietary antioxidant vitamins and death from coronary heart disease in postmenopausal women. *N Engl J Med* 1996; **334**: 1156–62.

10 Marchioli R. Antioxidant vitamins and prevention of cardiovascular disease: laboratory, epidemiological, and clinical trial data. *Pharmacol Res* (in press).

11 Steinberg D, and workshop participants. Antioxidants in the prevention of human atherosclerosis. *Circulation* 1992; **85**: 2337–44.

12 Steinberg D. Antioxidants and atherosclerosis: a current assessment. *Circulation* 1991; **84**: 1420–25.

13 Steinberg D, Parthasarathy S, Carew TE, Khoo JC, Witztum JL. Beyond cholesterol: modifications of low-density lipoprotein that increase its atherogenicity. *N Engl J Med* 1989; **320**: 915–24.

14 The Alpha-Tocopherol, Beta Carotene Cancer Prevention Study Group. The effect of vitamin E and beta carotene on the incidence of lung cancer and other cancers in male smokers. *N Engl J Med* 1994; **330**: 1029–35.

15 Stephens NG, Parsons A, Schofield PM, et al. Randomised controlled trial of vitamin E in patients with coronary disease: Cambridge Heart Antioxidant Study (CHAOS). *Lancet* 1996; **347**: 781–86.

16 Meydani M, Natiello F, Goldin B, et al. Effect of long-term fish oil supplementation on vitamin E status and lipid peroxidation in women. *J Nutr* 1991; **121**: 484–91.

17 Gruppo Italiano Studio sulla Sopravvivenza nell'Infarto miocardico (GISSI). Il protocollo dello studio GISSI-Prevenzione: Studio di

intervento preventivo sulle componenti aterosclerotica e trombotica del rischio post-infarto. *G Ital Cardiol* 1993; **23**: 1053–61.
18 Santoro E, Franzosi MG, Nicolis E. A computerized network system for the management of a large-scale multicentre clinical trial: the GISSI-3 trial. *Control Clin Trials* 1993; **14**: 430.
19 Graziani MS, Ceriotti F, Carobene A, et al on behalf of SIBioC Prevenzione Group. Accuracy of cholesterol measurements in Italian clinical laboratories: joint project GISSI-Prevention Italian Society of Biochemistry. *Eur J Clin Chem Clin Biochem* 1997; **35**: 311–15.
20 Stampfer MJ, Buring JE, Willett W, Rosner B, Eberlein K, Hennekens CH. The 2X2 factorial design: its applications to a randomized trial of aspirin and carotene in U.S. Physicians. *Stat Med* 1985; **4**: 111–16.
21 Byar DP, Piantadosi S. Factorial designs for randomized clinical trials. *Cancer Treat Rep* 1985; **69**: 1055–62.
22 Marubini E, Valsecchi MG, eds. Analysing survival data from clinical trials and observational studies. Chichester, UK: John Wiley, 1995.
23 De Vita C, Franzosi MG, Geraci E, et al. GISSI-2 mortality plus extensive left ventricular damage as "end-points". *Lancet* 1990; **335**: 289.
24 Ascherio A, Rimm EB, Stampfer MJ, Giovannucci EL, Willett WC. Dietary intake of marine n-3 fatty acids, fish intake and the risk of coronary disease among men. *N Engl J Med* 1995; **332**: 977–82.
25 Albert CM, Hennekens CH, O'Donnell CJ, et al. Fish consumption and risk of sudden cardiac death. *JAMA* 1998; **279**: 23–28.
26 Kromhout D, Bosschieter EB, de Lezenne CC. The inverse relationship between fish consumption and 20-year mortality from coronary heart disease. *N Engl J Med* 1985; **312**: 1205–09.
27 Daviglus ML, Stamler J, Orencia AJ, et al. Fish consumption and the 30-year risk of fatal myocardial infarction. *N Engl J Med* 1997; **336**: 1046–53.
28 Dolecek TA. Epidemiological evidence of relationships between dietary polyunsaturated fatty acids and mortality in the Multiple Risk Factor Intervention Trial. *Proc Soc Exp Biol Med* 1992; **200**: 177–82.
29 Rodriguez BL, Sharp DS, Abbott RD, et al. Fish intake may limit the increase in risk of coronary heart disease morbidity and mortality among heavy smokers: The Honolulu Heart Program. *Circulation* 1996; **94**: 952–56.
30 De Lorgeril M, Salen P, Martin J-L, Monjaud I, Delaye J, Mamelle N. Mediterranean diet, traditional risk factors, and the rate of cardiovascular complications after myocardial infarction: final report of the Lyon Diet Heart Study. *Circulation* 1999; **99**: 779–85.
30 Singh RB, Rastogi SS, Verma R, et al. Randomised controlled trial of cardioprotective diet in patients with recent acute myocardial infarction: results of one year follow up. *BMJ* 1992; **304**: 1015–19.
32 McLennan PL. Relative effects of dietary saturated, monounsaturated, and polyunsaturated fatty acids on cardiac arrhythmias in rats. *Am J Clin Nutr* 1993; **57**: 207–12.
33 McLennan PL, Bridle TM, Abeywardena MY, Charnok JS. Dietary lipid modulation of ventricular fibrillation threshold in the marmoset monkey. *Am Heart J* 1992; **123**: 1555–61.
34 Kang JX, Leaf A. Antiarrhythmic effects of polyunsaturated fatty acids: recent studies. *Circulation* 1996; **94**: 1774–80.
35 Billman GE, Kang JX, Leaf A. Prevention of ischemia-induced ventricular arrhythmias by dietary pure n-3 polyunsaturated fatty acids in dogs. *Circulation* 1999; **99**: 2452–57.
36 Sellmayer A, Witzgall H, Lorenz RL, Weber PC. Effects of dietary fish oil on ventricular premature complexes. *Am J Cardiol* 1995; **76**: 974–77.
37 Christensen JH, Gustenhoff P, Ellersen E, et al. n-3 fatty acids and ventricular extra systoles in patients with ventricular tachyarrhythmias. *Nutr Res* 1995; **15**: 1–8.
38 Christensen JH, Korup E, Aaroe J, et al. Fish consumption, ω-3 fatty acids in cell membranes, and heart rate variability in survivors of myocardial infarction with left ventricular dysfunction. *Am J Cardiol* 1997; **79**: 1670–73.
39 Siscovick DS, Raghunathan TE, King I, et al. Dietary intake and cell membrane levels of long-chain ω-3 polyunsaturated fatty acids and the risk of primary cardiac arrest. *JAMA* 1995; **274**: 1363–67.
40 Kang JX, Leaf A. Effects of long-chain polyunsaturated fatty acids on the contraction of neonatal rat cardiac myocytes. *Proc Natl Acad Sci USA* 1994; **91**: 9886–90.
41 Kang JX, Leaf A. Prevention and termination of the β-adrenergic agonist-induced arrhythmias by free polyunsaturated fatty acids in neonatal rat cardiac myocytes. *Biochem Biophys Res Commun* 1995; **208**: 629–36.
42 Kang JX, Xiao Y-F, Leaf A. Free, long-chain, polyunsaturated fatty acids reduce membrane electrical excitability in neonatal rat cardiac myocytes. *Proc Natl Acad Sci USA* 1995; **92**: 3997–4001.
43 Xiao Y-F, Kang JX, Morgan JP, Leaf A. Blocking effects of polyunsaturated fatty acids on Na+ channels of neonatal rat ventricular myocytes. *Proc Natl Acad Sci USA* 1995; **92**: 1100–04.
44 Xiao Y-F, Wright SN, Wang JK, Morgan JP, Leaf A. n-3 fatty acids suppress voltage-gated Na+ currents in HEK293t cells transfected with the α-subunit of the human cardiac Na+ channel. *Proc Natl Acad Sci USA* 1998; **95**: 2680–85.
45 Xiao Y-F, Gomez AM, Morgan JP, Lederer WJ, Leaf A. Suppression of voltage-gated L-type Ca2+ currents by polyunsaturated fatty acids in adult and neonatal rat cardiac myocytes. *Proc Natl Acad Sci USA* 1997; **94**: 4182–87.
46 Marchioli R, Bomba E, Tognoni G. Sheffield risk and treatment table for cholesterol lowering in prevention of coronary heart disease. *Lancet* 1996; **347**: 467–68.
47 Mitchinson MJ, Stephens NC, Parsons A, Blight E, Schoefield PM, Brown MJ. Mortality in the CHAOS trial. *Lancet* 1999; **353**: 381.
48 Ness A, Davey Smith G. Mortality in the CHAOS trial. *Lancet* 1999; **353**: 1017–18.
49 Rapola JM, Virtamo J, Ripatti S, et al. Randomised trial of alpha-tocopherol and beta-carotene supplements on incidence of major coronary events in men with previous myocardial infraction. *Lancet* 1997; **349**: 1715–20
50 MacMahon S, Peto R, Cutler J, et al. Blood pressure, stroke, and coronary heart disease, part 1, prolonged differences in blood pressure: prospective observational studies corrected for the regression dilution bias. *Lancet* 1990; **335**: 765–74.
51 Collins R, Peto R, MacMahon S, et al. Blood pressure, stroke, and coronary heart disease, part 2, short-term reductions in blood pressure: overview of randomised drug trials in their epidemiological context. *Lancet* 1990; **335**: 827–38.
52 Diaz MN, Frei B, Vita JA, Keaney JF. Antioxidants and atherosclerotic heart disease. *N Engl J Med* 1997; **337**: 408–16.
53 Marchioli R, Tognoni G. Beneficial effects of statins. *Lancet* 1996; **348**: 1542.
54 Vaughlam CJ, Murphy MB, Buckley BM. Statins do more than just lowering cholesterol. *Lancet* 1996; **348**: 1079–82.
55 Princen HMG, van Duyvenvoorde W, Buytenhek R, et al. Supplementation with low doses of vitamin E protects LDL from lipid peroxidation in men and women. *Arterioscler Thromb Vasc Biol* 1995; **15**: 325–33.
56 Suzukawa M, Ishikawa T, Yoshida H, Nakamura K. Effect of in-vivo supplementation with low-dose vitamin E on susceptibility of low-density lipoprotein and high-density lipoprotein to oxidative modification. *J Am Coll Nutr* 1995; **14**: 46–52.
57 Weber P, Bendich A, Machlin LJ. Vitamin E and human health: rationale for determining recommended intake levels. *Nutrition* 1997; **13**: 450–60.
58 Porkkala-Sarataho EK, Nyyssonen MK, Kaikkonen JE, et al. A randomized, single-blind, placebo-controlled trial of the effects of 200 mg α-tocopherol on the oxidation resistance of atherogenic lipoproteins. *Am J Clin Nutr* 1998; **68**: 1034–41
59 De Waart FJ, Moser U, Kok FJ. Vitamin E supplementation in elderly lowers the oxidation rate of linoleic acid in LDL. *Atherosclerosis* 1997; **133**: 255–63.
60 Omenn GS. What accounts for the association of vegetables and fruit with lower incidence of cancers and coronary heart disease? *Ann Epidemiol* 1995; **5**: 333–35.